Light Scattering Study of Ovomucin

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The pH, ionic strength, temperature, and time dependence of the weight average molecular weight, M_w, of ovomucin, the structural glycoprotein of chicken egg white, has been determined by light scattering. Ovomucin is aggregated at neutral pH; at pH 6.2 and 7.9, M_w is 240×10^6 and 40×10^6, respectively. In 6.5 *M* guanidine hydrochloride, M_w is 23×10^6. These M_w data indicate the disaggregation of ovomucin is an important step in the process by which egg white "thins." Under conditions of minimum aggregation, 0.15 ionic strength and pH 11.5, the M_w of ovomucin was still observed to decrease. This alkaline degradation appears to be due to disulfide bond splitting by hydroxide ion. The rate constant for degradation obtained from scattering measurements (1×10^{-5} sec^{-1}) was equal, within experimental error, to that obtained from the decrease in cystine content measured by amino acid analysis (1.4×10^{-5} sec^{-1}). The degradation appeared to be first order with respect to hydroxide ion activity, and had an activation energy of 32 ± 5 kcal/mol. The ratio $R_g/M_w^{1/2}$ remained constant during degradation, indicating that ovomucin retains a random coil conformation during hydrolysis.

Ovomucin, the glycoprotein responsible for the gel-like structure of avian egg white, is not sufficiently soluble at neutral pH in nondenaturing solvents so that its molecular weight and conformation can be determined. Lanni *et al.* (1949) obtained a molecular weight of 7.6×10^6 from sedimentation–viscosity measurements on a soluble fraction of ovomucin which contained the virus hemagglutination inhibitor of egg white. Molecular weights of 28×10^6 for human urinary mucoprotein (Maxfield, 1961) and 8×10^6 for porcine submaxillary mucin (Bettelheim and Dey, 1965) have been reported. Although these glycoproteins have a much larger carbohydrate content than ovomucin (Donovan *et al.*, 1970; Maxfield, 1966; Bettelheim and Dey, 1965), structural similarities to ovomucin are possible. Other studies of ovomucin have been carried out on samples which have been chemically modified with mercaptoethanol (Robinson and Monsey, 1964; Donovan *et al.*, 1970; Kato *et al.*, 1971), dithiothreitol (Dam and Hill, 1966), and thioglycolate or peroxide (Nakamura *et al.*, 1969).

Characterization of modified material provides information about modified ovomucin molecules, but physical measurements on the intact glycoprotein are required to determine how ovomucin functions as the structural protein of egg white. Knowledge of the molecular weight and conformation is necessary to understand the process by which egg white thins. Although ovomucin is more soluble at higher pH, the occurrence of alkaline degradation necessitates time-dependent studies in order to obtain data characteristic of native ovomucin. Donovan *et al.* (1972) studied the rate of change of viscosity of ovomucin as a function of concentration, pH, and temperature. They observed that the reaction producing the viscosity decrease is first order with respect to both ovomucin concentration and hydroxide ion activity, and has an activation energy of about 7 kcal/mol. Since light scattering is particularly suited for the study of systems undergoing rapid changes in weight average molecular weight, we have used this technique to follow the alkaline degradation of ovomucin as a function of pH, ionic strength, and temperature. If it is assumed that scattering data obtained at finite times and angles can be extrapolated reliably to zero time and zero angle, then the extrapolated scattering data yield values for molecular weight, radius of gyration, and virial coefficient which are representative of ovomucin in its native state. These measurements, together with molecular weight estimates in 6.5 *M* guanidine·HCl and 0.1 *N* NaOH, indicate that native ovomucin at acid or neutral pH is an aggregated, flexible coil polymer which undergoes both disaggregation and covalent bond hydrolysis at alkaline pH.

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MATERIALS AND METHODS

Britton–Robinson universal buffer mixtures (Britton and Robinson, 1931), prepared with reagent grade chemicals (except for USP grade diethylbarbituric acid), were used in the rate studies. The measured pH was corrected for temperature (Britton and Welford, 1937). Guanidine·HCl was prepared from commercial (Eastman P736) guanidine carbonate by the method of Nozaki and Tanford (1967). Guanidine·HCl concentration was calculated from solution density (Kawahara and Tanford, 1966).

Preparation of Ovomucin. Details of the procedure used to prepare ovomucin have been described (Donovan *et al.*, 1972). To remove lysozyme from the ovomucin solutions used in the present studies, the ovomucin was dispersed in 0.1 *M* KCl, pH 4.9, and Biogel CM P-50 was added. The Biogel with bound lysozyme was separated by settling or by low speed centrifugation, leaving lysozyme-free ovomucin in the supernatant (pH 6.2, 0.1 *M* KCl). The supernatant was either diluted with an equal volume of 2 *M* KCl or used directly as stock solution for scattering measurements. Other stock solutions were prepared in the same manner except that the pH was adjusted to 7.9 with 1 *N* NaOH. Stock solutions were centrifuged at about half-speed in a clinical centrifuge for 5 min to sediment a small amount of particulate matter, and the supernatant was stored at 4° until used. The higher ionic strength enhanced ovomucin stability, and no additional particulate matter was formed over a period of several weeks. However, no stock solution was used for longer than 2 weeks after preparation. Ovomucin concentrations of the stock solutions were determined from absorbance at 277.5 nm ($E_{1\,cm}^{1\%} = 9.3$), corrected for scattering (Donovan *et al.*, 1970). Lysozyme concentrations of these solutions, as measured by assay using *Micrococcus lysodeikticus* (Garibaldi *et al.*, 1968), was less than 0.4% by weight of the ovomucin concentration.

Light Scattering. Scattering measurements were made with a Sofica photometer, using $\lambda = 436$ nm. Calibration of this instrument and the necessary corrections for reflection effects

have been described (Tomimatsu *et al.*, 1968). Rate studies were carried out as follows. Since sintered glass filters cannot withstand strongly alkaline solutions, alkali-resistant Millipore filters were used to clarify the buffers. These filters were preconditioned by passing buffer through them until a low constant solvent scattering was obtained. Approximately 10 ml of buffer was filtered into a weighed scattering cell containing a small magnetic stirrer. The cell was reweighed and the volume of filtered buffer solution was calculated from the density of the buffer. Angular measurements were made on the buffer (after temperature equilibration for runs at temperatures different from ambient, 23°) and then, with the cell in place, a suitable aliquot of ovomucin stock solution was added from a calibrated pipet. Upon addition of the aliquot of ovomucin stock solution to the solvent, there was no significant change in the negligible number of scintillations (due to dust particles) seen at low angles of observation. The buffer was stirred magnetically during the addition, which required about 15 sec. Zero time was taken to be the time when addition of the aliquot was completed. Stirring was continued for an additional 15 sec. Angular measurements were taken continuously for the first hr and less frequently during the second hr. A series of 11 angles were read in 5 min, starting with the smallest angle, 30°, since the rate of change in scattering was faster at the forward angles. The procedure was repeated at least three times for each set of conditions, using different aliquots of the ovomucin stock solution so that time-dependent angular scattering data were obtained for at least three concentrations. Ovomucin concentrations were calculated from the measured volumes of buffer and ovomucin stock solution used.

Scattering measurements in 6.5 *M* guanidine·HCl were carried out in a similar manner; no time-dependent change in scattering was observed. Measurements were also made on a freeze-dried sample of lysozyme-free ovomucin (Donovan *et al.*, 1970) dissolved in 0.1 *N* NaOH. After 102 hr at room temperature, the nonturbid solutions were clarified using alkali-resistant Millipore filters. The solutions showed a slow, continuing decrease in scattering of about 0.3% per hr. This slow change was assumed to be due to a slow degradation which was not hydrolysis of disulfide bonds (see below, Figures 3, 4, and 5). Since this degradation probably starts at zero time, the M_w observed at 102 hr was increased by 31% to correct for this slow continuing degradation.

Refractive Increment, d*n*/d*c*, at 436 nm. Differences in refractive index between solvent and sample solutions were measured with a Brice (Brice and Halwer, 1951) differential refractometer. The refractive increment for ovomucin was determined on the freeze-dried sample (Donovan *et al.*, 1970) dissolved in 0.1 *N* NaOH. Ovomucin concentrations were calculated from the measured dry weight of sample used. The refractive increment measured in 0.1 *N* NaOH was also applied to scattering measurements in Britton–Robinson buffers. Small corrections (Hughlin, 1965) to d*n*/d*c* were applied to measurements at ionic strength 1.1 and at temperatures which differed appreciably from ambient. The refractive increment for ovomucin in 6.5 *M* guanidine·HCl was calculated using the Lorenz and Lorentz formulation (Hughlin, 1965; Rupp and Mommaerts, 1957) in which d*n*′/d*c* is

$$dn'/dc = (dn/dc) + \bar{v}(n_0 - n_0') \quad (1)$$

the refractive increment in 6.5 *M* guanidine·HCl, d*n*/d*c* is the refractive increment in 0.1 *N* NaOH, $\bar{v}$ is the partial specific volume of ovomucin estimated from chemical analysis (Donovan *et al.*, 1970) to be 0.715 ml/g, and n_0 and n_0' are the refractive indices of 0.1 *N* NaOH and 6.5 *M* guanidine·HCl, respectively. The refractive index at $\lambda = 436$ nm of 6.5 *M* guanidine·HCl was calculated to be 1.452 by extrapolation of refractive index data at $\lambda = 589$ nm given by Kielley and Harrington (1960). The dispersion equation of Perlmann and Longsworth (1948) was applied to convert refractive index data obtained at 589 nm to 436 nm.

Loss of Cystine on Alkaline Hydrolysis. Ovomucin was incubated in a pH-stat at pH 11.45, 39.9°, 0.1 ionic strength. Samples were withdrawn at known times and placed in HCl to stop the degradation reaction. Hydrolysis of the protein and amino acid analysis were carried out as described previously (Donovan and White, 1971). The pH measurement was made at room temperature.

Treatment of Scattering Data. For time-dependent studies, the raw scattering data at each angle were plotted against time and smooth curves were drawn through the experimental points. Intensities at a given time were read from these curves and corrected for reflection effects. Weight average molecular weight (M_w), radius of gyration (R_g), and second virial coefficient *B* were calculated from the Rayleigh–Debye (Kerker, 1969) approximation

$$Kc/R_\theta = 1/M_wP(\theta) + 2Bc \quad (2)$$

where *c* is ovomucin concentration in grams per milliliter, θ is the angle of measurement, R_θ is Rayleigh's ratio, $K = 2\pi^2n^2(dn/dc)^2/\lambda_0^4N_A$ is the light scattering constant, and $P(\theta)$ is the particle scattering factor. A computer program (Pickett, 1967) utilizing the Zimm (1948) method of analysis was applied to evaluate the light scattering parameters, M_w, R_g, and *B*. All computations were done on an IBM 1800 computer.

RESULTS

Refractive Increment, d*n*/d*c*, at $\lambda = 436$ nm. Measurements on four different ovomucin concentrations in 0.1 *N* NaOH gave d*n*/d*c* = 0.182 ± 0.001 ml/g, which compares with 0.184 ml/g for ovomucoid (Tomimatsu *et al.*, 1966); these two egg white glycoproteins have comparable total carbohydrate (Donovan *et al.*, 1970; Warner, 1954). For ovomucin in 6.5 *M* guanidine·HCl, the calculated d*n*′/d*c* of 0.102 ml/g (see Materials and Methods Section) is for no preferential binding of a solvent component by ovomucin (Timasheff and Kronman, 1959). If preferential binding of guanidine·HCl occurs and is comparable to the average 5.5% binding of guanidine·HCl to various proteins observed by Hade and Tanford (1967), then the molecular binding treatment of Timasheff and Kronman (1959) shows that d*n*′/d*c* would be increased to 0.107 ml/g. Bettelheim (1971) reported a refractive increment of 0.122 ml/g at $\lambda = 546$ for human submaxillary saliva glycoprotein in 6.0 *M* guanidine·HCl.

Light Scattering Measurements. The results of scattering measurements in 0.1 *N* NaOH and 6.5 *M* guanidine·HCl are summarized in Table I. The observed weight average molecular weight in 0.1 *N* NaOH of 2.2×10^5 can be compared with the number average value of 1.1×10^5 and the viscosity average value of 1.6×10^5 observed in 6 *M* guanidine·HCl − 0.2 *M* mercaptoethanol (Donovan *et al.*, 1970). The molecular weight in 6.5 *M* guanidine·HCl of 23×10^6 is that of ovomucin molecules which are disaggregated, but which have all covalent bonds intact (Tanford *et al.*, 1967). If the refractive increment is not corrected for preferential binding of salt (as discussed above), M_w in 6.5 *M* guanidine·HCl would be increased to 25×10^6.

Results of a typical time-dependent experiment at pH 11.74, ionic strength 0.15, and 23.3° are shown in Figure 1, which

Table I. Light Scattering Parameters for Ovomucin Observed under Various Conditions of Measurement

Ovomucin stock solution	Measurement conditions				Light scattering parameters[a]			
	Solvent	pH	Temp, °C	Ionic strength	$M_w \times 10^{-6}$	R_g (Å)	$B \times 10^4$ mole-cm³/g²	Curve[b]
c	0.1 *N* NaOH	...	23.3	...	0.22	...	...	...
0.1 *M* KCl, pH 6.2	6.5 *M* guanidine·HCl	7.10	23.3	...	23	2300	0	...
1.1 *M* KCl, pH 7.9	Universal buffer	11.26	23.3	0.15	56	2900	0	A
1.1 *M* KCl, pH 7.9	Universal buffer	11.74	23.3	0.15	46	2910	0.5	B
1.1 *M* KCl, pH 7.9	Universal buffer	12.24	23.3	0.15	39	2590	1.0	C
1.1 *M* KCl, pH 7.9	Universal buffer	11.78	17.2	0.15	40	2600	0.1	D
1.1 *M* KCl, pH 7.9	Universal buffer	11.57	28.1	0.15	27	2250	1.2	E
1.1 *M* KCl, pH 7.9	Universal buffer + 1 *M* KCl	11.72	23.3	1.1	41	2240	0.1	F
1.1 *M* KCl, pH 6.2	Universal buffer	11.52	23.3	0.15	270	5960	0	G
0.1 *M* KCl, pH 6.2	Universal buffer	11.55	23.5	0.10	210	5650	0.1	H

[a] Except for the first two samples, all values are for extrapolated zero time data (see Methods and Materials Section). [b] See Figures 3, 4, and 5. [c] Freeze-dried sample (Donovan *et al.*, 1970).

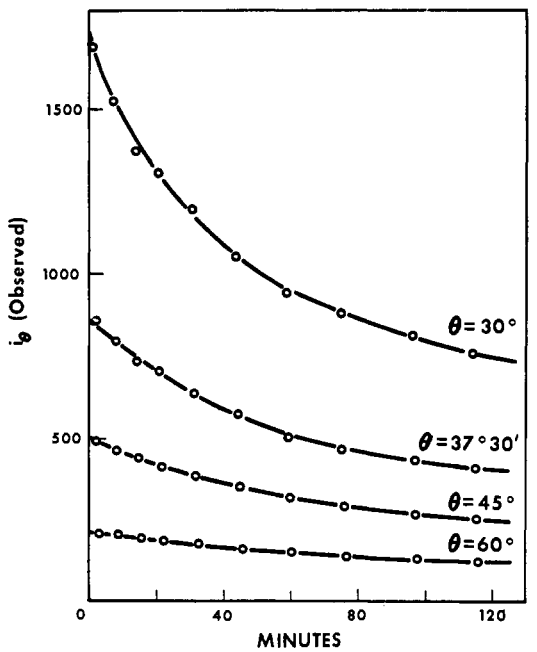

Figure 1. Time dependence of scattered light intensity as a function of angle. Conditions: 4.77×10^{-5} g/ml of ovomucin, pH 11.74, 23.3°, ionic strength 0.15, pH 7.9 ovomucin stock solution

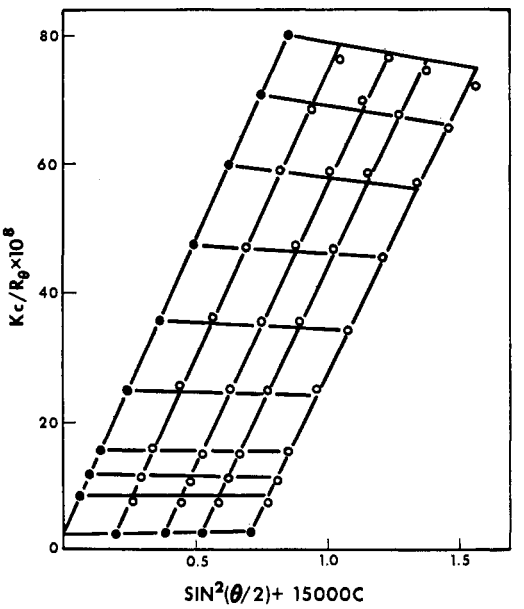

Figure 2. Zimm plot of zero time scattering data for ovomucin at pH 11.74, 23.3°, ionic strength 0.15, pH 7.9 ovomucin stock solution. Solid lines are computer-calculated least-squares lines (see text), open circles are experimental points, and filled circles are extrapolated points

depicts the change in intensity with time for selected forward angles. For intermediate angles, smaller changes were observed; at the larger backward angles the changes were larger than for intermediate angles but still much less than for the smaller forward angles. A Zimm plot of the extrapolated zero time data for the same experiment is shown in Figure 2. The computer program used (Pickett, 1967; Tomimatsu *et al.*, 1968) expands Kc/R_θ in a two-dimensional power series and makes a least-squares fit on a surface in Kc/R_θ, $\sin^2 \theta/2$, and *c* space, rather than the set of lines of a Zimm plot. Therefore entire sets of experimental points may appear on the same side of the least-squares line, *e.g.*, points for constant angle in Figure 2. Data at 142°, 30′ and 150°, which tended to show convex curvature, indicating molecular weight polydispersity (Kerker, 1969), were not included in the computer least-squares analysis. Since the Zimm plot intercept (see Figure 2) is very close to zero, a small uncertainty in the intercept corresponds to a large uncertainty in M_w. Therefore, on an absolute basis, the M_w's may not be very accurate. However, for a given set of conditions, the experimental time-dependent data are obtained on the same sample solution so that the precision of the observed changes in M_w should be good and the data suitable for kinetic treatment. This is shown to be so by the small scatter of the points in Figures 1 and 2 above and Figures 3 to 6 below. Zero-time light scattering parameters obtained with pH 6.2 and pH 7.9 ovomucin stock solutions are listed in Table I. The large difference in M_w (210–270 $\times 10^6$ for the pH 6.2 stock solution compared to 27–56 $\times 10^6$ for the pH 7.9 stock solution) suggests that disaggregation of ovomucin takes place as the solution pH becomes further removed from the isoionic pH of 4.75 calculated for ovomucin (Donovan *et al.*, 1970). A similarly large difference is observed for R_g values. The virial coefficient, *B*, is small in all cases. However, *B* values for the pH 7.9 stock solution do show the trend expected for a polyelectrolyte as a function of increasing pH and temperature, *i.e.*, *B* becomes larger as the net charge or the temperature increases (Tanford, 1961).

To minimize complications due to concurrent disaggregation, the pH 7.9 stock solution of ovomucin was used in the study of alkaline degradation. Time dependent changes in M_w and R_g as a function of pH are shown in Figure 3; the

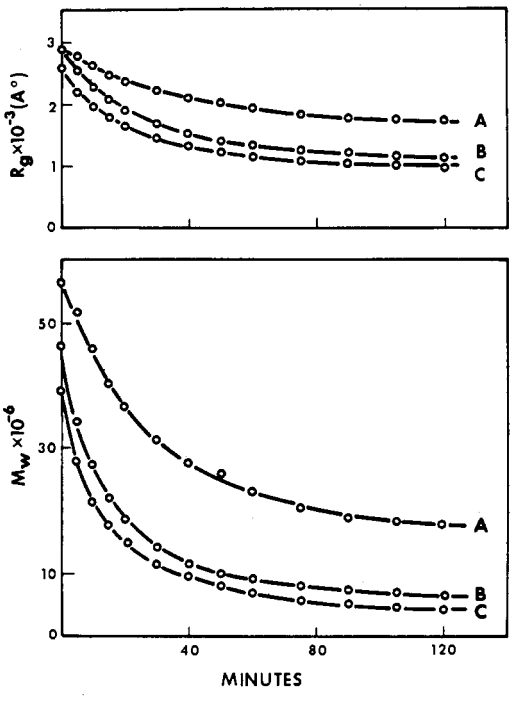

Figure 3. Time dependence of M_w and R_g as a function of pH. Conditions: 23.3°, ionic strength 0.15, pH 11.26 (curve A), pH 11.74 (curve B), and pH 12.24 (curve C). Ovomucin stock solution, pH 7.9. Zero time points are extrapolated values

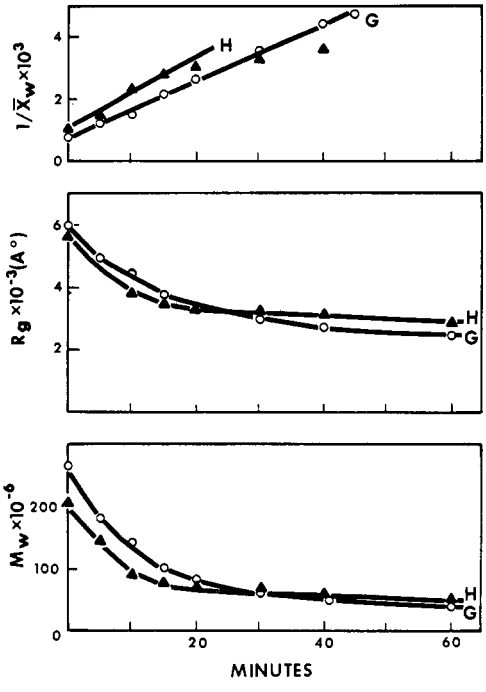

Figure 5. Time dependence of M_w, R_g, and $1/\bar{X}_w$ of highly aggregated ovomucin. Conditions: pH 11.52, 23.3°, ionic strength 0.15 (curve G) and pH 11.55, 23.5°, ionic strength 0.10 (curve H). Ovomucin stock solution, pH 6.2. Zero time points are extrapolated values

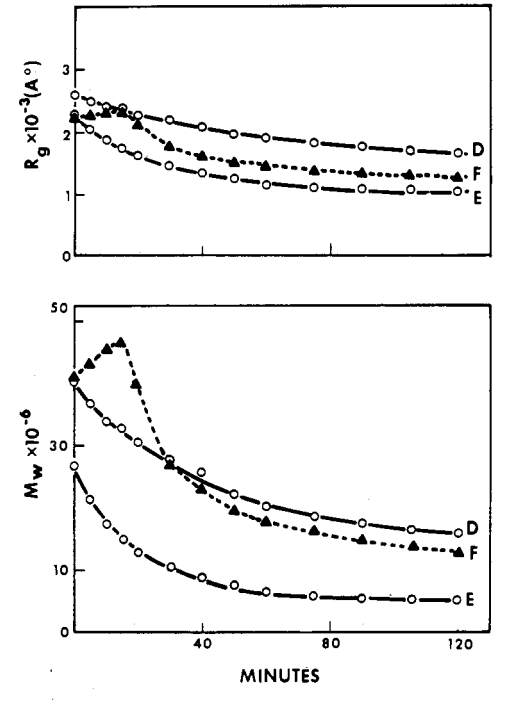

Figure 4. Time dependence of M_w and R_g as a function of temperature and ionic strength. Conditions: pH 11.78, ionic strength 0.15, 17.2° (curve D); pH 11.57, ionic strength 0.15, 28.1° (curve E); and pH 11.72, 23.3°, ionic strength 1.1 (curve F). Ovomucin stock solutions, pH 7.9. Zero time points are extrapolated values

effects of temperature and ionic strength are shown in Figure 4. Both M_w and R_g decrease monotonically with time, and more rapidly at higher pH (Figure 3). The rate of change in M_w and R_g is also greater at higher temperature (Figure 4). Only at higher ionic strength is there an irregular change—both M_w and R_g increase initially, then decrease in a regular manner (Figure 4). Figure 5 shows the decrease in M_w and R_g which results when highly aggregated ovomucin at pH 6.2 is brought to pH 11.5. In this case, the rate of change in M_w and R_g is initially faster at the lower ionic strength, but after 20 min the rate of change becomes faster at the higher ionic strength.

Loss of Cystine. The ratio half-cystine/aspartic acid (Donovan and White, 1971), obtained as a function of time of alkaline hydrolysis of ovomucin at pH 11.45, ionic strength 0.10, at 39.9° was 0.565, 0.547, 0.487, and 0.432 at 0, 15, 30 and 60 min, respectively. The rate constants calculated from these data, which appear to be fit by first-order kinetics, are 8.2×10^{-5} sec^{-1} (k_1) and 2.9×10^{-2} M^{-1} sec^{-1} (k_2). The latter value is slightly lower than that observed for the hydrolysis of the disulfide bonds of ovomucoid (Donovan and White, 1971). In order to calculate the rate constants at 23.3°, for comparison with the rate constants observed by light scattering (see Table II), an activation energy of 20 kcal/mol was assumed for alkaline hydrolysis (Donovan and White, 1971).

DISCUSSION

The data in Table I indicate that ovomucin has an M_w of about 240×10^6 at pH 6.2, and about 40×10^6 at pH 7.9. Since the M_w obtained in 6.5 M guanidine·HCl is 23×10^6 (Table I), ovomucin is highly aggregated at pH 6.2 and only partially aggregated at pH 7.9. An exact interpretation of the time dependence of the light-scattering data requires consideration of two processes, disaggregation and degradation. However, the degree of aggregation is small at pH 7.9, and changes in M_w with time for this stock solution at alkaline pH can be interpreted, without significant error, in terms of the degradation process alone.

We shall assume that disaggregation and degradation take place by random chain separation and random chain scission, respectively. If the molecular weight remains large, *i.e.*, if the number of chains separated or bonds broken is small com-

pared to the total number of chains or bonds, then only the initial stages of the reaction are involved and the light scattering results can be interpreted. The reciprocal of the weight average degree of polymerization, $1/\bar{X}_w$, at time t, is then given by (Tanford, 1961):

$$1/\bar{X}_w = 1/(\bar{X}_w)_0 + k_1 t/2 \quad (3)$$

The subscript 0 refers to the initial degree of polymerization and k_1 is the pseudo-first-order rate constant. For the disaggregation process, the "monomer" molecular weight, M_0, is taken as 23×10^6; for the degradation step, M_0 has been assumed equal to the molecular weight measured in 0.1 N NaOH (2.2×10^5, see Table I). In either case, $M_w = M_0\bar{X}_w$.

Data obtained with the pH 7.9 ovomucin stock solution (Figures 3 and 4) were used to calculate rate constants for the degradation process by use of eq 3. Plots of $1/\bar{X}_w$, shown in Figure 6, were initially a linear function of time, a necessary condition (Tanford, 1961) for applying eq 3. The linear portions of the $1/\bar{X}_w$ curves encompass molecular weights which range from those for the partially aggregated systems at zero time to M_w's which are smaller than the monomer molecular weight for the disaggregation process. Therefore, a single process, degradation, appears to account for the time-dependent changes in M_w observed in experiments with pH 7.9 ovomucin stock solution (Figures 3 and 4). The pseudo-first-order rate constants calculated from the plots are given in Table II. (For curve F in Figure 6, the linear portion between 15 and 30 min was used to estimate k_1.) The second-order rate constants presented in Table II were obtained by dividing the first-order rate constants by hydroxide ion activity.

Interpretation of the molecular weight decrease which takes place when the pH 6.2 stock solution of ovomucin is used (Figure 5) is simplified if one of the two processes, disaggregation or degradation, is much faster than the other. If disaggregation ($M_0 = 23 \times 10^6$) is assumed to account for the M_w changes represented by curves G and H, then the rate of disaggregation is 3.7×10^{-4} sec^{-1} and 4.8×10^{-4} sec^{-1}, respectively. On the other hand, if the aggregates ($M_w = 270 \times 10^6$ for curve G and $M_w = 210 \times 10^6$ for curve H) are considered to be large molecules which undergo degradation only ($M_0 = 2.2 \times 10^5$), then $k_1 = 3.0 \times 10^{-6}$ sec^{-1} and 3.9×10^{-6} sec^{-1}, respectively, for curves G and H (see Figure 5 and Table II). These latter values for k_1 are smaller but not too different from those obtained for the pH 7.9 ovomucin stock solution for which degradation is the primary process. In fact a smaller value for k_1 for the pH 6.2 ovomucin stock solution can be readily explained. If the aggregates of random coils (see below) have multiple aggregation points for each monomer ($M_0 = 23 \times 10^6$), hydrolysis of a bond will cause a decrease in M_w of a monomer, but may not cause a decrease in M_w of an aggregate, since the remaining points of aggregation would prevent chain separation. We conclude that: *at pH 11.5, the rate of disaggregation is much slower than the rate of degradation.* However, from extrapolation of the kinetic data at higher pH (Table II), the rate constant for degradation at pH 7.9 is calculated to be about 10^{-9} sec^{-1} at 23°, so that after 2 weeks at 4° (maximum storage time and temperature of stock solutions), degradation is expected to cause only a small change (less than 1%) in M_w. Thus, the observed difference in M_w between pH 6.2 and 7.9 ovomucin stock solutions is largely due to disaggregation, *i.e., disaggregation is faster than degradation at pH 8.*

Because of the similar decrease in molecular weight observed when ovomucin is exposed to alkali and to dithiothreitol, Donovan *et al.* (1971) suggested that disulfide bond hydrolysis is the degradation reaction. The data presented here appear to confirm this suggestion. For loss of half-cystine at 23.3°, the calculated rate constant, 13.5×10^{-6} sec^{-1} (see Table II), is about twice that observed by light scattering at the same pH. However, chemical analysis for cystine as a function of time would give a larger rate constant if ovomucin has disulfide bond loop structures or multiple disulfide bonds (three or more) for each unit of $M_w = 2.2 \times 10^5$ (see below). Hydrolysis of a disulfide bond in such a loop structure or in a multiply-bonded portion of the molecule would cause no decrease in M_w but would be detected by chemical analysis. For ovomucin, the pH-dependent (curves A, B, and C), temperature-dependent (curves B, D, and E), and ionic strength-dependent (curves B and F) data in Table II indicate that the degradation reaction is approximately first

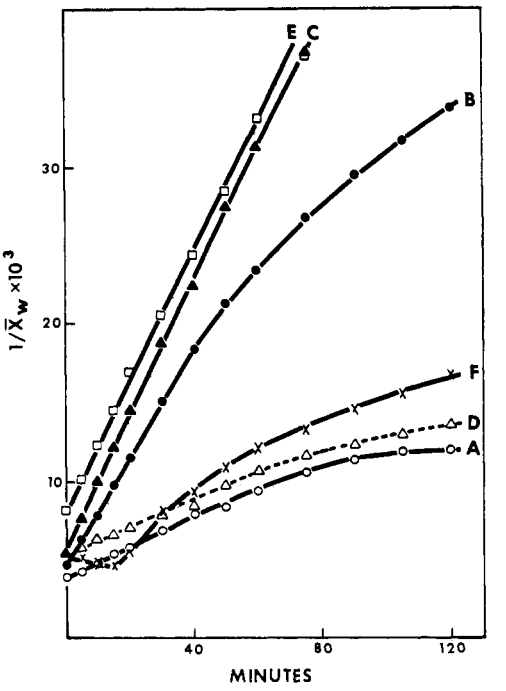

Figure 6. Time dependence of $1/\bar{X}_w$ as a function of pH, temperature, and ionic strength. Conditions: 23.3°, ionic strength 0.15, pH 11.26 (curve A), pH 11.74 (curve B), and pH 12.24 (curve C); pH 11.78, ionic strength 0.15, 17.2° (curve D); pH 11.57, ionic strength 0.15, 28.1° (curve E); pH 11.72, 23.3°, and ionic strength 1.1 (curve F). Ovomucin stock solution, pH 7.9. Zero time points are extrapolated values

Table II. Pseudo-First-Order and Second-Order Rate Constants for Alkaline Degradation of Ovomucin

Curve[a]	pH	Temp, °C	Ionic strength	$k_1 \times 10^6$ (sec^{-1})	$k_2 \times 10^3$ (M^{-1} sec^{-1})
A[b]	11.26	23.3	0.15	3.4	1.9
B[b]	11.74	23.3	0.15	11.4	2.1
C[b]	12.24	23.3	0.15	14.9	0.9
D[b]	11.78	17.2	0.15	3.0	0.5
E[b]	11.57	28.1	0.15	13.9	3.7
F[b]	11.72	23.3	1.1	7.5	1.4
G[c]	11.52	23.3	0.15	3.0	0.9
H[c]	11.55	23.5	0.10	3.9	1.1
d	11.45	23.3[d]	0.10	13.5	4.8

[a] See Figures 5 and 6. [b] pH 7.9 ovomucin stock solution. [c] pH 6.2 ovomucin stock solution. [d] Data from cystine analysis; experimental temperature was 39.9°; k_1 and k_2 at 23.3° are calculated values (see text).

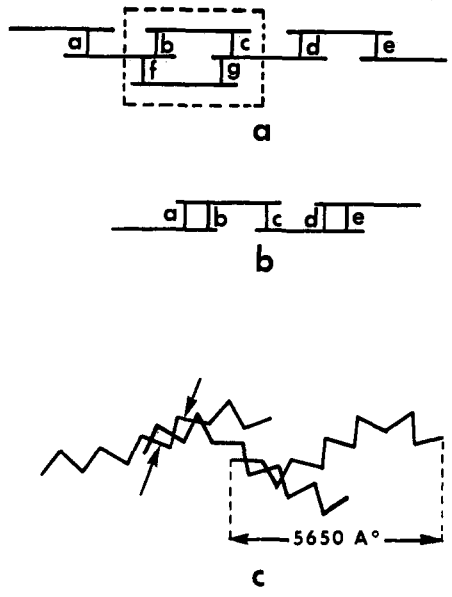

Figure 7. Diagrammatic representation of ovomucin (not to scale): a and b, parts of ovomucin chain; c, aggregated ovomucin (see text)

order with respect to hydroxide ion activity, has an activation energy of 32 ± 5 kcal/mol, and is only slightly dependent on ionic strength. For ovomucoid (Donovan and White, 1971), the degradation reaction (disulfide bond hydrolysis) is first order with respect to hydroxide ion activity, has an activation energy of 20 kcal/mol, and accelerates with increase in ionic strength. Hydrolysis of the disulfide bonds of ovomucoid is accompanied by conformational changes (Donovan and White, 1971); ovomucin largely maintains its random coil conformation during degradation, since the ratio $R_g/M_w^{1/2}$ remains essentially constant (see Figures 3, 4, and 5).

The initial increase in M_w at ionic strength 1.1 (Figure 4) is difficult to explain, but is probably not associated with a conformational change, since $R_g/M_w^{1/2}$ remains constant. It may be that at high ionic strength there is an enhancement of the specific effect of buffer ions on aggregation (Kronman *et al.*, 1964), so that transfer of ovomucin from 1.1 *M* KCl, pH 7.9, to ionic strength 1.1, pH 11.72, in the presence of Britton–Robinson buffer components results in aggregation.

The electron microscope picture presented by Donovan *et al.* (1970) suggests that native ovomucin is associated in a branching arrangement, with chain widths ranging from 20 to 100 Å and with overall lengths greater than 10,000 Å. Carbohydrate side chains (Donovan *et al.*, 1970) and lateral association of ovomucin molecules probably account for the large observed chain widths. Maxfield (1961) reported a length of 12,000 Å and a width of 80 Å for urinary mucoprotein. The present light scattering results on ovomucin are consistent with these dimensions. For example, measurements at pH 11.55, 23.5°, and ionic strength 0.10 gave M_w and R_g of 210 × 10^6 and 5650 Å, respectively (see Table I). The calculated root-mean-square end-to-end distance for a random coil with R_g equal to 5650 Å is 13,800 Å, which is in the range observed in the electron micrograph (Donovan *et al.*, 1970). The length of an equivalent stiff rod is 19,600 Å. This latter value, together with M_w of 210 × 10^6 and the partial specific volume for ovomucin of 0.715 ml/g (Donovan *et al.*, 1970), gives 130 Å as the expected diameter of the ovomucin molecule. This high value for diameter can be brought into better agreement with that observed in the electron micrograph by taking into account the effect of branching on R_g. Following Zimm and Stockmayer (1949), we take six as the average number of branch units with at least four segments per branch. The latter quantity is estimated from the minimum branch length seen in the electron micrograph (Donovan *et al.*, 1970), and the calculated (Tanford, 1961) effective segment, β, of 530 Å which gives a segment length, l, of 175 Å for ovomucin from $\beta = 3l$ (Tanford, 1961). For such a branching arrangement R_g is reduced to about one-half of the unbranched value (Zimm and Stockmayer, 1949). Correcting for this effect we calculate an expected diameter of about 90 Å, in better agreement with the electron micrograph results (Donovan *et al.*, 1970). From the electron micrograph alone one can not establish whether the branches are covalent or are the result of aggregation. Similar calculations made with data obtained in 6.5 *M* guanidine·HCl give 70 Å as the expected diameter without any correction for branching. Since there is no aggregation in 6.5 *M* guanidine·HCl (Tanford *et al.*, 1967), it appears that the branches observed in the electron micrograph are the result of aggregation.

Hydrolysis of bonds cross-linking chains which have lengths comparable to that of the polymer would result in little change in R_g but a large change in M_w so that $R_g/M_w^{1/2}$ would increase rapidly as degradation proceeded. The experimental observation that $R_g/M_w^{1/2}$ remains constant during degradation indicates that ovomucin is composed of cross-linked chains which are *short*, compared to the length of the undegraded polymer. Figure 7 is a schematic representation of ovomucin structure as revealed by our experiments. Figure 7a shows part of an ovomucin chain composed of units with M_w = 2.2 × 10^5 (horizontal lines) which are cross-linked by disulfide bonds (vertical lines a–g). Hydrolysis of bonds a, d, or e decreases M_w but hydrolysis of one cross-linking bond in the loop structure (dashed-line box) will not result in chain separation unless one of the other bonds in the box is also hydrolyzed. In multiply-bonded units (shown in Figure 7b), hydrolysis of bond c separately will decrease M_w but hydrolysis of bonds a, b, d, or e separately causes no change in M_w. These loop structures and multiply-bonded units should contribute to chain rigidity and may account for the large β (530 Å) observed for ovomucin. The cystine content of ovomucin [35 disulfide bonds per 2.2 × 10^5 g (Donovan *et al.*, 1970)] provides ample opportunity for the existence of loop structures and multiply-bonded units.

Aggregated ovomucin monomers are depicted in Figure 7c. The monomer has a root-mean-square end-to-end distance (calculated from R_g in 6.5 *M* guanidine·HCl, Table I) of 5650 Å, with an effective segment length (represented by the straight line segments) of 530 Å. Hydrolysis at points indicated by the arrows causes a large change in M_w of disaggregated chains, but would cause no change in M_w of the aggregate if it were maintained by large contact areas between chains as illustrated. Figure 7c also shows how branching can originate. The lack of an extensive network (see Figure 2 of Donovan *et al.*, 1970) suggests that aggregation is not completely random, but might be the result of association at some specific portions of the ovomucin chains. Here we distinguish between aggregation and association according to Kronman and Andreotti (1964), in the sense that *association* refers to *specific* interactions, whereas *aggregation* refers to *nonspecific* interactions. If specific association could occur between random coils, this might account for an apparent slow disaggregation rate even when the electrostatic repulsion is large, as at pH 11.5. Alternatively, the slow disaggregation rate may be a diffusion-controlled separation of regions of highly tangled chains.

These light scattering measurements offer revealing insights into the decrease in viscosity of egg white (usually referred to as "thinning") observed on storage of eggs. The time-dependent changes in M_w of aggregated ovomucin which take place at pH 11.5 can be accounted for by the alkaline degradation process alone, suggesting that, at this pH, disaggregation is a much slower process than degradation. However, since the degradation process is very slow at pH 7.9, the observed difference in M_w of ovomucin at pH 6.2 and 7.9 (270×10^6 and 40×10^6) is probably due to disaggregation. Since thinning occurs between pH 7.6 and 9.7 (Sharp and Powell, 1931), much of the thinning might be produced by disaggregation of gel-like aggregates of ovomucin in the thick portion of the egg white, rather than by alkaline degradation of disulfide bonds. It appears possible to resolve this question by other physical techniques, and such studies are now underway in this laboratory.

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Water Vapor Sorption Hysteresis in Dehydrated Food

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Determination of the 40 and 100°F adsorption and desorption isotherms of a variety of dehydrated foods revealed the presence and extent of hysteresis as a characteristic of food type, represented by the sorption patterns of apple, pork, and rice. The integrals of hysteresis, obtained by plotting desorption–adsorption values at water activity intervals of 0.05 for these foods were, respectively, at 100°F: 0.08, 0.70, and 2.13; and at 40°F: 2.79, 0.90, and 3.72. Distribution of hysteresis along the isotherm had a distinctive pattern. The effect of increase in temperature was to decrease the amount of hysteresis and to limit its extent along the isotherm. The effect of storage was to increase the area of the hysteresis loop, due mainly to a decrease of the adsorptive capacity of the material. These findings suggest the possibility of using the changes of hysteresis as an index of quality deterioration.

The key to understanding the water sorption properties of food is the water vapor sorption isotherm, which is schematically represented in Figure 1. The shape of the isotherm reflects the manner in which the water is bound. Up to a water activity, A_w, of about 0.30, where the first inflection appears, water is held on polar sites of relatively high energies. This is the "monomolecular region." Between about A_w 0.30–0.70 there is a "multi-layer region." Above A_w 0.7 the water approaches the condition of "condensed water"; it is relatively free and the isotherm reflects solution and surface capillary effects.

The moisture sorption isotherm has many practical as well as theoretical applications in food. Rockland (1969) re-

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